UNITED STATES DEPARTMENT OF AGRICULTURE

PROCEDURE FOR DETERMINING THE TOTAL BETA-AMYLASE IN BARLEY, GREEN MALT, AND DRIED MALT[1]

Reagents

Citrate buffer solution: 0.2 M, pH 6.4 - 6.6.

Solution a: Dissolve 82.016 gm. of c.p. citric acid ($C_6H_8O_7.H_2O$) in 400 cc. of carbonate-free 1.0 N NaOH, add 1 cc. of toluene for preserving, and dilute to 1 liter. The pH of this solution should be 4.96.

Solution b: Mix 530 cc. of solution "a" with 470 cc. of 0.2 N NaOH. The pH of this solution should be about 6.6.

Phosphate buffer solution: 0.1 M, pH 8.3. Dissolve 14.2 gm. of c.p. anhydrous Na_2HPO_4 in CO_2 free distilled H_2O, add 1 cc. of toluene, and make up to 1 liter.

Cysteine-HCl solution: Dissolve 18.75 gm. of c.p. cysteine-HCl in distilled H_2O and make up to 500 cc. Twenty cubic centimeters of this solution should require 4.5 cc. of 1.0 N NaOH for neutralization.

Procedure

For barley: Pipette 20 cc. of cysteine-HCl solution into a 200 cc. volumetric flask and neutralize with 4.5 cc. of 1.0 N NaOH, add 50 cc. of citrage buffer solution "b" and make up to 200 cc. with distilled H_2O. Test this solution with brom-thymol blue on a spot plate. The pH should not exceed the range of 6.4 to 6.7.

Weigh out 10 gm. of finely ground barley into a 250 cc. Erlenmeyer flask. To this, add 750 mg. of standard strength papain and approximately 15 gm. of 60 mesh alundum. To this mixture add a small portion of the buffered cysteine solution to obtain a pasty consistency. Swirl the flask until all lumps are broken up. Add the remainder of the extraction solution, stopper the flask, and immerse in a water bath at 20° C. This extract should be agitated at intervals of 15 to 20 minutes. After 2 1/2

1/ The procedure outlined and the data given herein were included in a paper entitled, "The Determination of Total Beta-Amylase in Barley, Green Malt, and Dried Malt", presented by S. R. Snider, of the Agricultural Marketing Service and the Bureau of Plant Industry, U.S.D.A., at the annual meeting of the American Society of Brewing Chemists, in New York City, May 24, 1940.

hours, filter the extract through 18 1/2 cm. S. and S. fluted filter paper. Cover the funnel with a watch glass to arrest oxidation and evaporation. Reject the first 50 cc. of the filtrate. If it is preferred to extract 25 gm. of barley the same ratio of reagents apply.

Pipette 20 cc. of the extract (or 10 cc. if the diastatic power exceeds 135° Lintner) into a 100 cc. volumetric flask and dilute to 100 cc. At this stage proceed according to the method of Anderson and Sallans (1937) for the determination of diastatic power by the ferricyanide method.

For malts: Proceed in the same manner as in the analysis of barley up to the filtration of the extract. Pipette three aliquots of 20 cc. or 10 cc. (as the case may require) into a 100 cc. volumetric flask and into two 100 cc. Erlenmeyer flasks. Immerse the volumetric flask in an ice water bath, preferably in a refrigeration unit and using lead rings so that the flask may be almost entirely immersed.

Predetermine electrometrically, on one of the aliquots, the amount of 0.1 N HCl necessary to bring the extract to pH 3.3. The progress of this titration may be checked by testing the solution on a spot plate with brom-phenol blue as pH 3.3 is approached, to avoid the necessity of several electrometric determinations.

When the temperature of the extract in the ice water bath is reduced to 0°C.-4° C., add the predetermined amount of 0.1 N HCl to the test solution and return the bath with the flask to the refrigerator for 15 minutes.

To the third aliquot, add the same amount of HCl as used in the other aliquots and determine electrometrically the amount of phosphate buffer solution necessary to restore the solution to pH 6.7.

Remove the test solution from the refrigerator after 15 minutes of the acid treatment and add the predetermined amount of phosphate buffer solution required for neutralization.

Dilute the solution to 100 cc. and proceed with the determination for diastatic power.

If desired, this solution may be tested on a spot plate with 3 to 5 drops of 5 percent solution of sodium nitroprusside and 5 drops of 10 percent ammonium hydroxide, for cysteine stability. If the purple color does not fade out completely in less than 10 minutes, the cysteine in the extract has not been oxidized to the extent that any inhibition of beta-amylase occurs.

Table 1.- Comparison of the relative activity of three commercial papains of vegetable origin

Experimental Sample No. 1

200 cc. of 5 percent extract for 20 hours at 20° C.

Papain				Cysteine-HCl (neutralized)	Total beta-amylase Degrees Lintner Method	
No.	Source	Amount	Activity units of Klim 1/		A.S.B.C.	FeCy
		Gm.		Gm.	Degrees	Degrees
1	Unknown	2	0.38	0	87.4	87.8
		2		1	82.8	85.4
		2		2	82.8	86.1
2	Ceylon	2	.41	0	86.2	88.9
		2		1	84.6	88.3
		2		2	82.8	89.4
3	Mexican	2	.06	0	66.1	71.5
		2		1	69.1	74.6
		2		2	72.1	76.5

1/ According to the method of Balls and Hoover (1937).

Table 2.- The effect of variable concentrations of barley and papain at variable time and temperature

Barley Sample No. 1

Test No.	Barley Gm.	Papain Gm.	Water cc.	Temperature Degrees C.	Time Hrs.	Toluene cc.	Degrees Lintner Degrees
1	25	3	150	20	24	4	89.2
2	25	4	200	20	24	4	88.9
38	10	2	200	20	24	4	90.5
4	10	2	200	20	24	-	89.5
5	10	2	200	20	48	-	88.2
6	10	2	200	20	48	4	87.6
7	10	4	200	20	24	-	88.0
8	10	3	200	20	24	-	88.6
9	10	2	200	20	24	-	89.5
10	10	1	200	20	24	-	87.5
11	10	.5	200	20	24	-	85.1
12	10	0	200	20	24	-	25.6
13	10	4	200	20	24	-	85.3
14	10	3	200	20	3	-	83.8
15	10	2	200	20	3	-	82.9
16	10	1	200	20	3	-	78.3
17	10	.5	200	20	3	-	72.9
18	10	0	200	20	3	-	24.0
19	10	4	200	40	3	-	86.5
20	10	2	200	40	3	-	89.4
21	10	1	200	40	3	-	80.8
22	10	.5	200	40	3	-	77.9

Table 3.- Stability of 1 percent solutions of cysteine-HCl and cysteine-HCl, neutralized.

Solution No.	Days standing before testing	Solution concentration in parts 1:100 Time 1/ Min.	1:100 Color reaction	1:1000 Time 1/ Min.	1:1000 Color reaction	1:10000 Time 1/ Min.	1:10000 Color reaction
1	0	6	Deep purple	6	Pink	6	Yellow
		17	Green brown	17	Yellow	-	-
		24	Yellow	-	-	-	-
2	1	7	Deep purple	7	Pink	7	Yellow
		16	Green brown	16	Yellow	-	-
		25	Yellow	-	-	-	-
3	3	6	Deep purple	6	Pink	2	Yellow
		15	Light brown	15	Yellow	-	-
		22	Yellow	-	-	-	-
4	20	5	Deep purple	5	Pink	-	-
		15	Light brown	15	Yellow	-	-
		21	Yellow	-	-	-	-
1a 2/	1	12	Dark brown	12	Yellow	-	-
		20	Yellow	-	-	-	-
2a 2/	3	12	Light brown	2	Yellow	-	-
		17	Yellow	-	-	-	-

1/ Spot plate test, reading after adding nitroprusside reagent.

2/ Solutions 1 and 2 respectively, neutralized.

Table 4.- The effect of activation of papain with cysteine in the extracting solution, 3 hours at 20° C.

Test No.	Papain	Cysteine-HCl	Citrate-buffer	pH extract	Nitro-prusside reaction	Diastatic power Degrees Lintner
	Gm.	Gm.				Degrees
1	2.0	2.0	0	5.8	Deep purple	88.6
2	1.0	1.0	0	5.8	do.	89.4
3	.8	.8	0	5.8	do.	91.2
4	.6	.6	0	5.8	Purple	92.3
5	.4	.4	0	5.8	do.	91.4
6	.2	.2	0	5.8	Faint purple	80.5
7	2.0	2.0	0.02 M	5.0	Deep purple	82.5
8	1.0	1.0	.02 M	5.0	do.	86.2
9	.8	.8	.02 M	5.0	do.	87.1
10	.6	.6	.02 M	5.0	Purple	85.1
11	.4	.4	.02 M	5.0	do.	86.0
12	.2	.2	.02 M	5.0	do.	86.4
13	2.0	.5	0	5.8	do.	84.9
14	1.0	.5	0	5.8	do.	87.8
15	.8	.5	0	5.8	do.	86.8
16	.6	.5	0	5.8	do.	87.3
17	.4	.5	0	5.8	do.	87.1
18	.2	.5	0	5.8	do.	86.8

Table 5._ Effect of the hydrogen ion concentration on the estimation of total beta-amylase in barley and malt extracts

Experimental Sample No. 1

10 gm.. extracted with 200 cc. solution for 3 hours at 20° C.
Extract Nos. 1, 2, and 3 contain 600 mg. of papain and 600 mg. of cysteine-HCl.
Extract Nos. 4, 5, and 6 contain 800 mg. of papain and 800 mg. of cysteine-HCl.

Extract No.	Buffer solutions									
	Molarity	Citrate pH 5.0 extract		Citrate pH 6.0 extract		Citrate pH 6.5 extract		Phosphate pH 7.0 extract		None pH 5.8 extract
		D.P.1/ °L.	pH	D.P.1/ °L.	pH	D.P.1/ °L.	pH	D.P.1/ °L.	pH	D.P.1/ °L.
1	0.02	85.1	5.0	86.4	5.6	89.7	6.2	84.7	6.7	89.5
2	.05	87.4	5.0	89.2	5.8	90.5	6.3	88.4	6.8	-
3	.10	-	-	89.2	5.9	90.1	6.4	89.4	6.9	-
4	.02	87.1	5.0	86.8	5.6	90.1	6.2	85.5	6.7	91.2
5	.05	-	-	87.1	5.8	90.8	6.3	88.5	6.8	-
6	.10	-	-	86.4	5.9	91.9	6.4	90.5	6.9	-

1/ Diastatic power, degrees Lintner.

Table 6. - The influence of phenylhydrazine on the reagents used in the extract for determining total beta-amylase

Experimental Sample No. 1

200 cc. of 5 percent extract for 20 hours at 20° C.

Extract No.	Papain 1/	Cysteine-HCl 2/	Solution concentration: Citrate buffer 3/	Solution pH	Phenylhydrazine 4/	Color reactions: Methylene-blue 5/	Nitroprusside	D.P. °L. 6/
	Mg.	Mg.						
1	600	600	0	-	0	Faint	Purple	86.6
2	600	600	.05 M	6.3	0	Gr. blue	do.	87.4
3	600	600	0	-	.05 M	Lt. blue	do.	86.2
4	600	600	.05 M	6.3	.05 M	Restored		88.5
5	600	0	0	-	0	Blue	-	86.3
6	600	0	.05 M	6.3	0	Blue	-	89.2
7	600	0	0	-	.05 M	Colorless	-	5.2
8	600	0	.05 M	6.3	.05 M	Colorless	-	35.7

1/ Dry powder added to ground barley

2/ Neutralized with N - NaOH to pH 6.5±.

3/ Final molarity of buffer in the extract.

4/ Final molarity of phenylhydrazine in the extract.

5/ 0.25 cc. of concentrated methylene-blue added. Color noted at the beginning and end of the extraction period.

6/ Diastatic power, degrees Lintner.

Table 7.- Comparison of total beta-amylase in barleys, determined by the modified method and by the old method

Variety	Old method		Modified method	
	D.P. - °L. 1/	$_pH$ extract	D.P. - °L. 1/	$_pH$ extract 2/
Atlas	79.8	5.5	84.0	6.5
Spartan	74.8	5.5	73.8	6.4
Velvet	109.6	5.6	110.0	6.5
Trebi	110.8	5.6	111.6	6.5
Oderbruker	157.6	5.5	164.0	6.4
Peatland	184.0	5.5	189.8	6.4

1/ Diastatic power in degrees Lintner.

2/ Extract buffered with citrate buffer, $_pH$ 6.7.

Table 8.- Comparison of the methods and application of the modified method in the analysis of green malts

Barley variety	Total beta-amylase green malt dry basis			Malting loss	Total beta-amylase Green malt corrected for malting losses to basis of barley			Barley	Variable		Alpha-amylase values
	Papain method 1/	Papain-cysteine method 2/		Steeping, germinating, and rootlet	Papain method 1/	Papain-cysteine method	Papain-cysteine method less alpha-amylase	Papain-cysteine method	Green malt vs. barley	Green malt total beta-amylase less alpha-amylase vs. barley	Modified Wohlgemuth method 3/
			Less alpha-amylase						Total beta-amylase		Green malt dry basis
	°L. 4/	°L. 4/	°L. 4/	Pct.	°L. 4/	°L. 4/	°L. 4/	°L. 4/	°L. 4/	°L. 4/	
Atlas	76.9	79.8	71.2	11.0	68.5	71.0	63.4	58.5	21.3	- 4.9	45.5
do.	89.5	93.2	86.5	11.0	79.7	82.9	76.2	79.4	13.8	- 3.2	48.8
do.	75.5	81.7	76.5	11.3	67.0	72.5	66.9	63.9	17.8	- 3.0	49.8
do.	168.6	180.6	130.0	10.7	151.2	161.3	116.3	122.5	58.1	- 6.2	77.8
do.	166.0	180.0	146.5	10.2	149.1	161.6	131.6	124.0	56.0	- 7.6	82.1
Velvet	259.6	272.0	201.1	11.0	222.1	244.0	179.0	180.7	91.3	- 1.7	114.7
Wisconsin 38	277.8	288.4	220.0	10.0	250.0	259.6	198.0	203.1	85.3	- 5.1	132.3
Manchurian	251.3	260.0	134.4	11.5	222.4	230.1	163.2	172.8	87.2	- 9.6	119.5
Odessa	224.0	228.7	183.2	9.3	203.2	207.4	166.2	151.1	77.6	15.1	105.4

1/ Five percent extracts with 1 percent solution of papain for 24 hours at 20° C.

2/ Modified method.

3/ Method of Ehrnst, Yakish, and Olson (1939).

4/ Degrees Linter.